SURVEY FOR CIGUATERA FISH POISONING IN WEST HAWAII

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Approximately 25–30 fishes have caused ciguatera poisoning in >100 individuals in Hawaii, as reported annually by the State Department of Health. Generally, about 6–10 species are involved including herbivores and carnivores. A specific site at Puako, on the island of Hawaii was selected because of persistent outbreaks of fish poisoning in the first few months of nearly every year due to *Cheilinus rhodochrous* (wrasse, po'ou). The survey consisted of (1) algae and *Gambierdiscus toxicus* assessment; (2) fish analysis by immunological assay; (3) following fish extraction testing in mouse toxicity assay; and (4) analysis with guinea pig atrium for an effect on Na^+ channels. The immunological assay showed borderline and positive in more than 50% of species examined (herbivores and carnivores). Several species of algae were found, including *Jania* sp. and *Turbinaria ornata* previously shown to be associated with *Gambierdiscus toxicus* blooms. In 5 areas along 2 miles of shoreline, *Gambierdiscus toxicus* was noted in 2 areas (9–291/g algae). Organic solvent extracts from *Ctenochaetus strigosus* and *Acanthurus sandvicensis* showed inhibition of the Na^+ channel in the guinea pig atrium assay. The inhibition appears to be very similar in action to tetrodotoxin.

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Approximately 115 incidents of ciguatera fish poisonings occurred in the South Kohala area from 1971–1992. The number of illnesses is highly underreported due to the flu-like symptoms of ciguatera (Hokama,1988) which often lead to its misdiagnosis by physicians. On Oahu, at a downtown restaurant in February 1991, approximately 21 individuals became ill (5 hospitalized) after consuming rose colored wrasses *Cheilinus rhodochrous* (po'ou). *Cheilinus rhodochrous* extracts have shown typical ciguatoxin-like activity in the immunological, mouse toxicity and guinea pig atrium analysis (Amra et al., 1990). The active toxins of the po'ou flesh extract were found in the 1:9 (methanol:chloroform) and 1:1 (methanol:chloroform) fractions in the silica gel chromatography analysis. The 10% methanol fraction contained 10M.U. in 100mg crude flesh extract, while the 50% methanol fraction had 5M.U. in 100mg crude flesh extract (1 M.U. (mouse unit), is the amount of fish extract that kills a 20g mouse in 24hrs). The origin of the implicated fish was an area called Puako on the South Kohala Coast of Hawaii (Fig.1).

Puako is on the leeward coast of Hawaii in the district of South Kohala. Before 1957 there was essentially no development along this coast. However, over the years many residential dwellings, a 3.5km accessible coastline road, boat launching ramp and several public right of ways have been developed. Although development has progressed markedly there are still no real beaches in this area. Coarse sand and gravel make up much of the ocean-land interface with its underlying structure composed mostly of lava. Therefore, the land tends to be very porous. Other nearshore areas are bordered mostly by *Prosopis pallida* (kiawe trees) and soil is sparse. The limited soil probably leads to nutrients being distributed in small, restricted quantities along the coast. However, as one nears the boat launching area, soil levels substantially increase which accounts for the often turbid nature of the water as one nears this location and this probably increases its nutrient content. Puako is an extremely arid region with a visible freshwater lens noticeable during the diving expeditions along the coast. This is the underground fresh water which tends to lower the salinity of the ocean water in some areas of Puako.

The Puako study area extends 3.4km from Puako Bay to approximately 1km N of Pauoa Bay; it was divided into areas A-D, each 850m long (Fig.1). The study commenced in April 1992 and visits were made every 2 months. Each visit included collection and identification of fish and

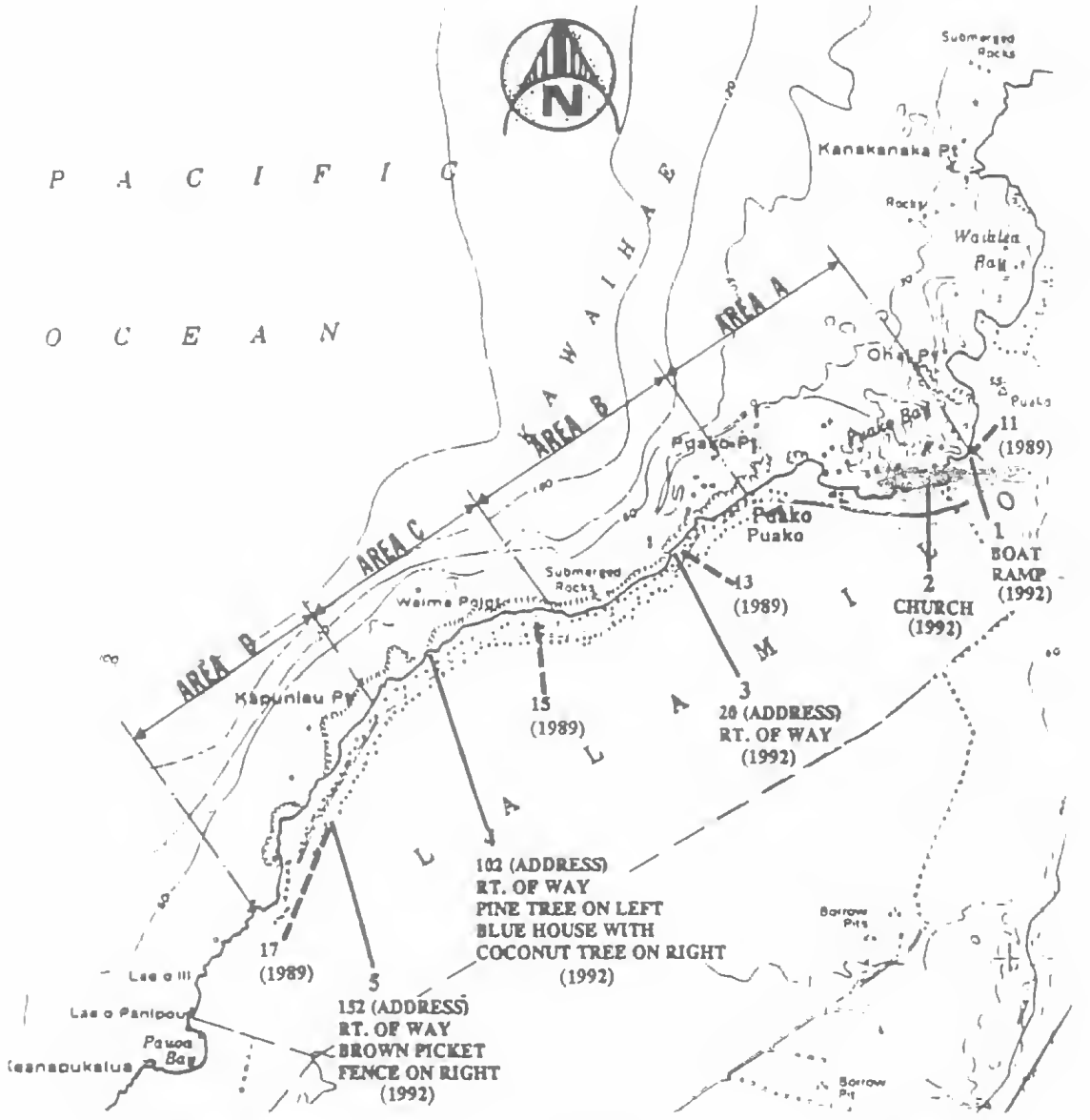


FIG.1. Map of the puako area surveyed for ciguatera. The 2 mile shoreline is transected into 4 segments (A, B, C and D) from north (A) to south (D).

algae, upon which were conducted: 1) identification of various algae types, 2) analysis of fish by the Solid Phase Immunobead Assay (S-PIA) for ciguatoxin and related polyether compounds, 3) analysis of algae for *Gambierdiscus toxicus*, 4) analysis of fish extracts with the mouse bioassay, 5) analysis of fish extracts with the guinea pig atrial assay, 6) water quality analysis. Part of the concept of this approach followed Yasumoto et al. (1980, 1984) and Hokama et al. (1993).

METHODS

IDENTIFICATION OF ALGAE

Algae from each station was placed into 50ml conical centrifuge tubes containing 2% formalin/seawater solution. Samples were taken to Professor Isabella Abbott (Department of Botany, University of Hawaii) for identification.

ANALYSES FOR *GAMBIERDISCUS TOXICUS*

Algal specimens were collected either by hand

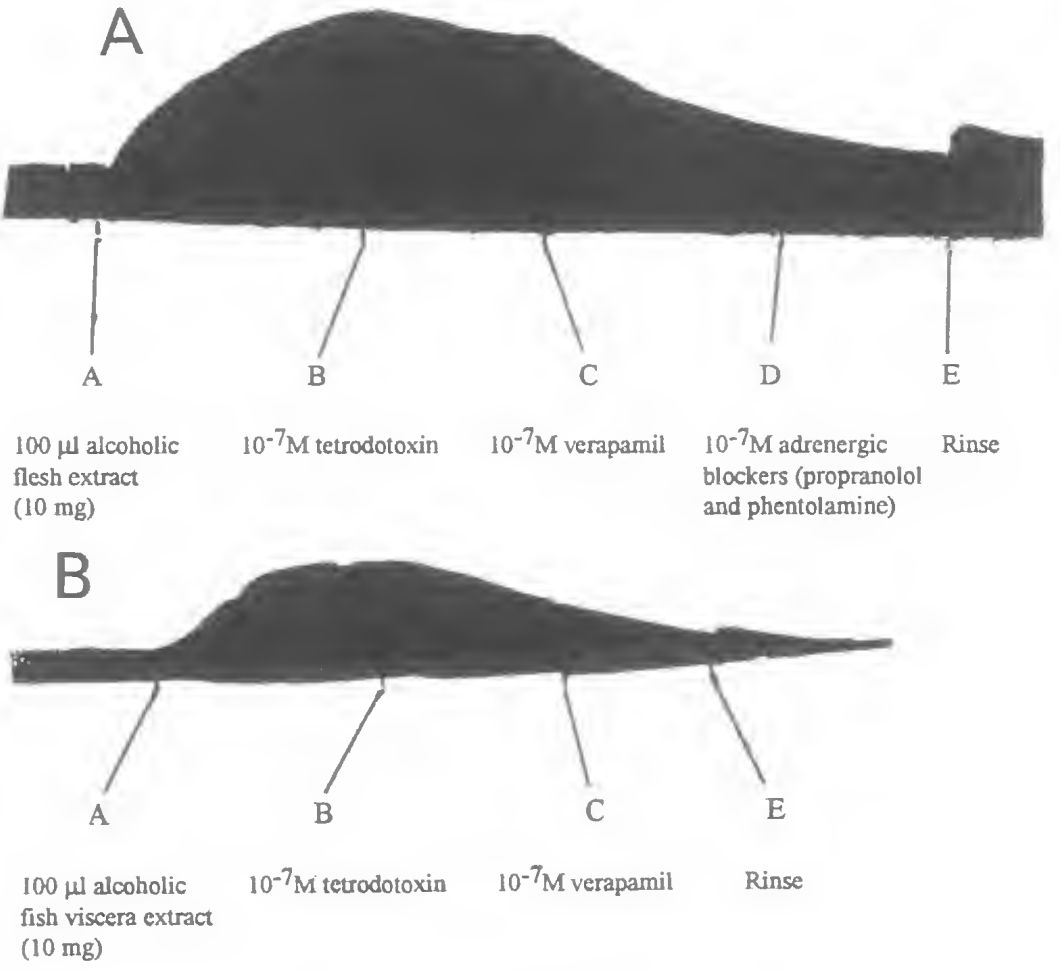
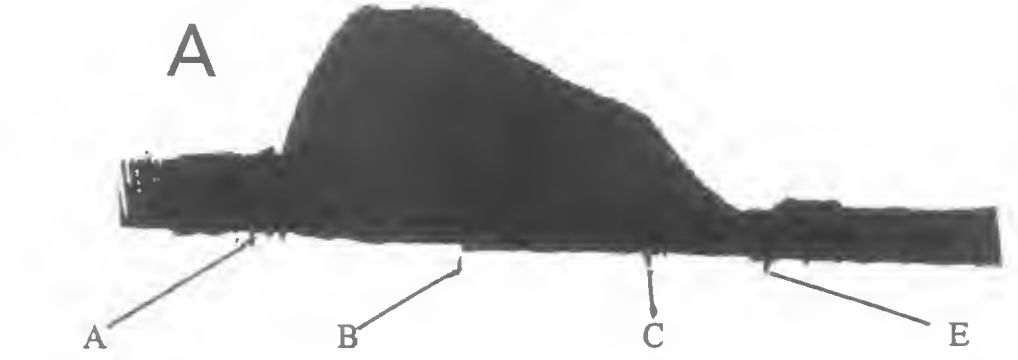


FIG.2. Guinea pig atrial responses to *Ctenochaetus strigosus* (kole) alcoholic extracts and the effects of blockers.

or by gently scraping coral substrates throughout areas A-D. Samples were then placed into 1 gallon ziplock bags containing 0.5–1l of seawater. The contents were shaken for 2min to remove any possible epiphytic dinoflagellates present on the algal substrate. The algae/seawater suspension was then consecutively passed over a 125µm mesh sieve to remove any large algal fragments then through a 38µm sieve. The residue on the 38µm sieve was then backwashed with an enriched seawater media, collected into a 100ml sterile glass bottle and loosely capped to provide aeration. After gentle agitation, 1ml was placed onto a Sedgewick Rafter Cell Counting Slide. Counts were performed in triplicate to determine the average number of cells per ml and the number of cells per gram of algae (Yasumoto et al.,1984).

Analysis of Fish by S-PIA

Various herbivorous and carnivorous fishes were collected by spearing and line fishing. A bamboo paddle coated with pentel correction fluid was then inserted into an incision made near the head of each sample and the residue retained on the paddle allowed to air dry for 5–10mins. The paddle was then immersed in methanol for 0.1sec and allowed to air dry. The paddle was then put into a blue latex bead-antibody solution (ciguatoxin antibody) for 5 or 10min intervals. Readings obtained were then scored according to the intensity of the coloring obtained on the pad-



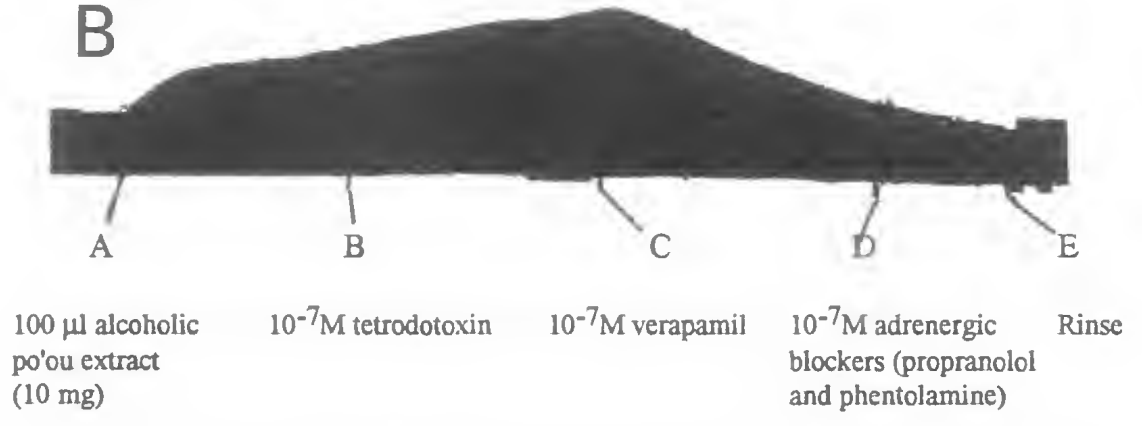


FIG.3. Guinea pig atrial responses to *Cheilinus rhodochrous* (po'ou) alcoholic extracts and the effects of blockers.

dle and rated as negative (no color), borderline (slight blue) or positive (distinct blue). The procedure followed Hokama (1990).

Extraction of Ciguatoxin and Related Polyether Compounds

Fish were separated into flesh and viscera samples and extracted for ciguatoxin and related polyether compounds. Each portion (flesh and viscera) was placed in acetone at a 1:2.5 ratio (flesh:acetone) and allowed to soak overnight. The acetone suspension produced was then decanted over a Whatman 4 filter and the filtrate allowed to collect into a flask. The acetone was then evaporated utilizing a Buchi Rotavapor. The residue retained in the flask was then washed with chloroform and partitioned with a brine solution (2x) at a 4:1 ratio (chloroform:brine) in a separatory funnel. The chloroform was successively evaporated and the residue retained was then brought up in a 80% methanol solution and partitioned with hexane (3x) at a 1:2 ratio (80% methanol:hexane). The 80% methanol was evaporated and the residue was collected and used for further analysis in the mouse bioassay and the guinea pig atrial assay. The method of Kimura et al. (1982), and Miyahara et al. (1989) were used in this study.

Analysis of Fish Extracts with Mouse Bioassay

100mg of the 80% methanol fraction fish extract was diluted in 1ml of a Tween 60/saline solution. The suspension was then injected intraperitoneally (IP) into a 20–25g Swiss Webster mouse and signs observed. Readings were successively taken at 30min, 1,2,4,6,8,24, and 48hrs post-injection and rated on a scale of 0–5 accord-

TABLE 1. Survey of *G. toxicus* at Puako, April to August, 1992. * areas refer to sections shown on Fig.1.

AREA *	DATE	*G. TOXICUS* CELLS/GM ALGAE	ALGAL SPECIES	TOTAL # FISH	S-PIA NEGATIVE	S-PIA BORDER LINE	S-PIA POSITIVE
A	APR 92	291	*Galaxaura fasciculata* *Tolypiocladia glomerulata*	74	18% (13)	55% (41)	27% (20)
	JUN 92	7.2	*Galaxaura marginata* *Galaxaura fasciculata*	76	43% (33)	43% (33)	13% (10)
	AUG 92	0	*Galaxaura marginata* with large clumps of *Biddulphia aurita* (diatom) *Galaxaura fasciculata* *Turbinaria ornata* epiphytic *Tolypiocladia glomerulata* *Dictyota friabilis* (brown) *Chondria polyrhiza, Jania* sp., *Cladophora* sp. (green)	86	30% (26)	63% (54)	7% (6)
B	APR 92	0	*Sargassum* sp.				
	JUN 92	0	*Turbinaria ornata* *Pterocladia caerulescens*				
	AUG 92	0	*Eupogodon iridescens* *Schizothrix calcicola* (bluegreen) *Centroceras clavulatum*				
C	APR 92	0	*Turbinaria ornata*				
	JUN 92	3.4	*Turbinaria ornata* with *Jania* *Pterocladia caerulescens*				
	AUG 92	0	*Schizothrix calcicola*				
D	APR 92	9.0	*Turbinaria ornata* with epiphytic *Jania*				
	JUN 92	1.2	*Turbinaria ornata* with *Jania* , bluegreen and *Biddulphia aurita* (diatom)				
	AUG 92	0	*Phormidium crosbyanum*				

ing to the signs presented. Toxicity ratings are scored according to Kimura et al. (1982).

Analysis of Fish Extracts with Guinea Pig Atrial Assay

Hartley Guinea Pig weighing approximately 300–350g were sacrificed and hearts surgically removed after anesthetization (*Enflurane*). The atria was then isolated from the ventricles and separated further into its left and right components. Each piece of atrial tissue was placed into a 25ml physiological bath (37°C) containing Krebs-bicarbonate solution and constantly aerated (95% O_2, 5% CO_2). Electrical probes stimulated the left atria while the right relied on its sinoatrial node. Subsequently, 100µl of fish extract (80% methanol fraction) at a concentration of 100mg/ml resuspended in Krebs bicarbonate solution was added to the physiological bath chamber. The tissue was then observed for both an inotropic and/or chronotropic responses displayed on a polygraph as reactions to the extract. Additionally, pharmacological characteristics were noted with the addition of 12.5µl of tetrodotoxin (sodium channel blocker), verapamil (calcium channel blocker) and propranolol/phentolamine (adrenergic blocking drugs) following the method of Miyahara et al. (1989). In one experiment (Fig.5) Manini extract from the viscera was used in the atrial analysis. The extract acted as a blocking agent. This action though resembling TTX or PSP was neither of these toxins, since it had a non-polar lipid characteristic.

Water Quality Analysis

Physical parameters of the Puako coastline (Areas A-D) were taken by the Department of Health Clean Water Branch in December 1989 and August 1992. Measurements including salinity, nitrate-nitrite, ortho-phosphates, ammonia, silica, total dissolved nitrogen, total dissolved phosphorous, temperature, conductance, dissolved oxygen and pH were taken utilizing a

TABLE 2. Puako fish assessment with S-PIA (April 1992). * Area refers to sections shown in Fig.1.

AREA *	SPECIES	TOTAL	S-PIA RESULTS		
			–	+/–	+
A	Hawaiin kole	1	0	1	0
	Kole	9	0	8	1
	Po'ou	6	2	3	1
	Roi	6	0	4	1
	Total	21	2	16	3
	Percent		(10)	(76)	(14)
B	Kole	9	1	4	4
	Po'ou	4	0	4	0
	Roi	2	0	0	2
	Total	15	1	8	6
	Percent		(7)	(53)	(40)
C	Hawaiin kole	6	0	6	0
	Kole	7	0	4	3
	Po'ou	4	1	2	1
	Roi	2	1	1	0
	Total	19	2	13	4
	Percent		(11)	(68)	(21)
D	Kaku *(S. barracuda)*	1	0	0	1
	Kole	10	0	10	0
	Po'ou	1	1	0	0
	Roi	7	2	4	1
	Total	19	3	14	2
	Percent		(16)	(74)	11
TOTAL (%)		74	8 (11)	41 (69)	15 (20)

Surveyor 2 water quality monitoring instrument. Testing areas for those parameters measured in December 1989 and August 1992 were within the areas of A through D (Fig.1).

RESULTS

IDENTIFICATION OF ALGAE

Puako contains a variety of algal types (Table 1), some of which have been found associated with *G. toxicus* previously.

ANALYSIS OF ALGAE FOR *G. TOXICUS* (Table 1)

The highest level of cells (per g of algae) occurred in area A during April 1992. *G. toxicus* was found at all stations (in limited quantities), except for Area B which was devoid of any dinoflagellates over the survey period. The moderate to high levels of *G. toxicus* were especially shown in April of 1992 in Area A and this was associated with *T. glomerulata*. Area B had no *G. toxicus* despite a variety of algae. Areas C & D had mild levels of *G. toxicus* probably associated with the epiphytic *Jania* sp.

TABLE 3. Puako fish assessment with S-PIA (June 1992). * Area refers to sections shown on Fig.1. # Not defined in Fig.1, but represents an area of similar size north of A. A is the first transect which originates at the boat ramp.

AREA	SPECIES	TOTAL	S-PIA RESULTS		
			–	+/–	+
A	Hawaiin kole	2	0	2	0
	Kole	6	6	0	0
	Po'ou	4	3	1	0
	Roi	3	0	2	1
	Total	15	9	5	1
	Percent		(60)	(33)	(7)
AA#	Hawaiin kole	1	1	0	0
	Kole	8	5	3	0
	Po'ou	3	3	0	0
	Roi	3	2	1	0
	Total	15	11	4	0
	Percent		(73)	(27)	(0)
B	Hawaiin kole	1	0	0	1
	Kole	7	3	4	0
	Po'ou	3	1	1	1
	Roi	4	0	2	2
	Table boss (spp.)	1	1	0	0
	Total	16	5	7	4
	Percent		(31)	(44)	(25)
C	Hawaiin kole	5	1	4	0
	Kole	2	0	2	0
	Po'ou	1	1	0	0
	Roi	3	1	2	0
	Total	11	3	8	0
	Percent		(27)	(73)	(0)
D	Hawaiin kole	5	5	0	0
	Kole	6	0	4	2
	Po'ou	4	0	3	1
	Roi	4	0	2	2
	Total	19	5	9	5
	Percent		(26.3)	(47.4)	(26.3)
TOTAL (%)		76	33 (43.4)	33 (43.4)	10 (13.2)

ANALYSIS OF FISH WITH S-PIA (Tables 2–4)

The targeted species in this Puako Survey included a herbivore *Ctenochaetus strigosus* (kole) and 2 carnivores *Cephalopholis argus* (roi);

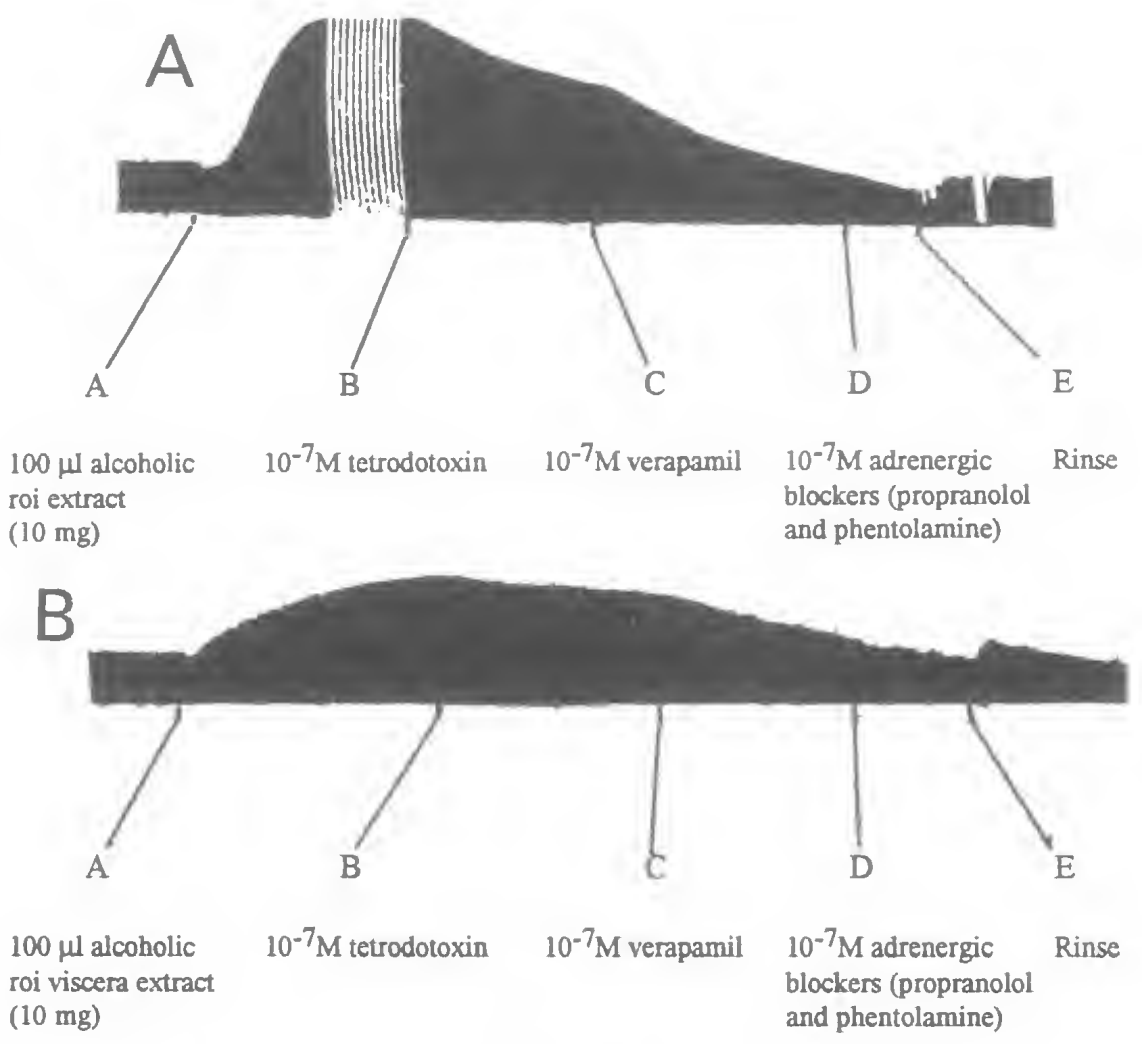


FIG.4. Guinea pig atrial responses to Cephalopholis argus (roi) alcoholic extracts and the effects of blockers.

Cheilinus rhodochrous (po'ou). The highest overall percentage of fish falling into the rejection category of S-PIA (borderline and positive) occurred during April (1992) in Areas A, B, and C. Area D had the greatest percentage of fish in the rejection range in August 1992.

ANALYSIS OF FISH EXTRACTS WITH THE MOUSE BIOASSAY (Table 5)

Both flesh and viscera contained compounds that induce abnormal symptoms and death in mice. Although all fish tested showed both high and low levels of toxicity, kole viscera on the average gave consistently the highest toxicity ratings. In all species, toxicity seemed to be higher in the viscera as compared to the flesh extract. There is a slight difference in the flesh and viscera toxicities in mice. The viscera extracts tended to be most consistently toxic and are noted in all areas and periods examined. The mouse toxicities of po'ou extracts from Areas A and D appear less severe than kole extracts. Again, within the species tested the viscera were more toxic especially in Areas B and C. Similarly, the roi extracts tended to be less toxic than the kole extracts. Again, the flesh extracts of roi appear to be less toxic than the roi viscera extract.

TABLE 4. Puako fish assessment with S-PIA (August 1992). * Area refers to sections shown on Fig.1. # Not defined in Fig.1, but represents an area of similar size north of AA, as described in Table 3.

AREA *	SPECIES	TOTAL	S-PIA RESULTS		
			–	+/–	+
A	Kole	20	6	12	2
	Papio	1	1	0	0
	Po'ou	3	2	1	0
	Roi	5	2	3	0
	Table boss	1	0	1	0
	Tang (*A. achilles*)	1	0	1	0
	Total	31	11	18	2
	Percent		(35.5)	(58.1)	(6.4)
B	Kole	10	3	7	0
	Po'ou	2	2	0	0
	Roi	3	1	2	0
	Total	15	6	9	0
	Percent		(40)	(60)	(0)
C	Kole	10	3	7	0
	Po'ou	2	1	1	0
	Roi	3	1	2	0
	Total	15	5	10	0
	Percent		(33)	(67)	(0)
D	Kole	10	0	6	4
	Po'ou	1	0	1	0
	Roi	4	0	4	0
	Total	15	0	11	4
	Percent		(0)	(73.3)	(26.7)
F#	Kole	10	4	6	0
	Total	10	4	6	0
	Percent		(40)	(60	(0)
TOTAL (PERCENT)		86	26 (30.2)	54 (62.8)	6 (7)

TABLE 5. Compilation of mouse toxicity of three species of fish: viscera and flesh for the months of April, June and August, 1992. * mouse toxicity values ranged from 1 (little or no toxicity) to 5 (not toxic). NS = no sample.

AREA	SPECIES	TISSUE	MOUSE RATING*		
			APR	JUN	AUG
A	*Ctenochaetus strigosus*	flesh	3	4	5
		viscera	5	5	5
B	*Ctenochaetus strigosus*	flesh	1	5	5
		viscera	5	5	5
C	*Ctenochaetus strigosus*	flesh	3	5	5
		viscera	5	5	5
D	*Ctenochaetus strigosus*	flesh	5	2	4
		viscera	5	5	4
A	*Cheilinus rhodochrous*	flesh	3	NS	3
		viscera	5	NS	3
B	*Cheilinus rhodochrous*	flesh	2	NS	5
		viscera	5	NS	5
C	*Cheilinus rhodochrous*	flesh	1	NS	4
		viscera	NS	NS	4
D	*Cheilinus rhodochrous*	flesh	1	NS	3
		viscera	NS	NS	3
A	*Cephalopholis argus*	flesh	2	3	5
		viscera	5	3	5
B	*Cephalopholis argus*	flesh	2	1	5
		viscera	4	2	5
C	*Cephalopholis argus*	flesh	1	2	5
		viscera	5	5	5
D	*Cephalopholis argus*	flesh	2	2	3
		viscera	4	5	NS

Analysis with the Guinea Pig Atrial Assay

Testing of fish extracts with the Guinea Pig atrial Assay indicated at least 2 pharmacologically different toxins. The inotropic effect elicited by one of these compounds is blocked by tetrodotoxin, indicating that the observed reaction has characteristics similar to ciguatoxin by allowing a greater influx of sodium ions through the membrane channel. Likewise, the second reaction observed is an inhibition by verapamil in response to the inotropic effect elicited by the fish extract. Both types of reactions (TTX and verapamil inhibitions) are found in the herbivores as well as the carnivores.

Inotropic patterns and their inhibition by tetrodotoxin, verapamil or both are demonstrated (Figs 2–4) for kule, po'ou and roi, respectively. Kole flesh and viscera extracts show typical inhibition by TTX and verapamil (Fig.2A,B). However, in some kole viscera extracts only verapamil or TTX showed inhibition.

Po'ou (whole fish) extract induced positive inotropy is inhibited by TTX and verapamil (Fig.3A). The latter inhibitor appears to give a stronger reaction. A pooled po'ou extract scored negative to the S-PIA test (negative for polyether); this is reflected by no inhibition with TTX channel blocker, but inhibition by verapamil(Figure 3b).

The major difference between the inotropic responses of whole roi (Fig.4A) and separated viscera extracts (Fig.4B) is in the initial rise. Both extracts are affected by TTX and verapamil, al-

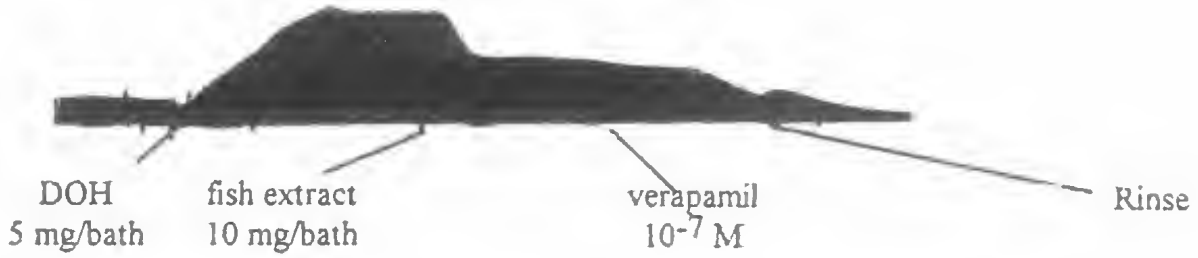


FIG.5. Inotropic response of pooled Department of Health (DOH) fish extract implicated in ciguatera poisoning and the negative inotropic effect of manini (*Acanthurus sandvicensis*). This DOH extract was toxic for mice (Value of 5) and its inotropic pattern showed strong block with tetrodotoxin (pattern not shown).

though the viscera extract appears to be affected less than the whole extract. There is a suggestion that the toxin in the viscera is slightly different from whole roi extract.

Fig.5 shows the inhibition of the inotropic effect of known ciguateric pooled fishes (Department of Health samples) by a sample from *Acanthurus* sp. extract (manini) giving a TTX or verapamil like block. This has been shown on occasion with kole extracts also.

Water Quality Analysis

The Puako area is associated with underground freshwater infusion into the ocean (Kay et al.,1977), especially in Areas B and C . Salinity has varied from a low of 2.2% to 3.4% in Areas B and C. This fluctuation in part may account for the variation in *G. toxicus* numbers.

DISCUSSION

There appears to be no relationship between high levels of toxicity in fish and presence or absence of *G. toxicus*; even in the absence of *G. toxicus*, levels of SPIA borderline and positive fishes were noted. April,1992 showed the least number of negative fish in all species and areas (18%) followed by August (30%) and then June (43%) (Tables 2,3,4). In general, the herbivores appeared to be more toxic (±and +) than the carnivores in all three months examined.

The mouse toxicity showed that the herbivore (kole) was the most toxic with higher number of mouse values of 5. The carnivores roi and po'ou were also toxic but less than the kole, except for the viscera extracts. Analysis of the guinea pig inotropic response suggested the ciguatoxin-like, maitotoxin-like (Figs 2,3,4) and a sodium channel blocking toxin (polyether-like) similar to TTX or verapamil (Fig.5).

It is difficult to correlate immunological results with extracts used in the mouse and atrial assays, since the immunoassay is carried out in individual fishes whereas the mouse and atrial assays are done with pooled extracts though of the same species. Nevertheless in general, those fishes with borderline and positive SPIA results appeared to be more toxic than the negative fishes.

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